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Award Number: DAMD17-01-1-0361

TITLE: Effects of Moderate Aerobic Exercise Combined with  
Caloric Restriction on Circulating Estrogens and IGF-I in  
Premenopausal Women

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| <b>13. ABSTRACT (Maximum 200 Words)</b><br><br>This proposal entitled "Effects of moderate aerobic exercise combined with caloric restriction on circulating estrogens and IGF-1 in premenopausal women" will provide important contributions regarding the primary prevention of breast cancer in women. This study has examined the effects of exercise training combined with caloric restriction, resulting in weight loss, on two hormonal biomarkers for breast cancer i.e., circulating estrogens and insulin-like growth factor I (IGF-I). As expected, exercise training 4 times per week combined with an 20-30% decrease in caloric intake over four menstrual cycles has produced significant increases in aerobic capacity (28-33%), weight loss ranging from 1.0 to 9 kg, and loss of body fat ranging from 5 to 12% of initial percent fat. Light conditioning resulted in significant gains in aerobic capacity (33%), but only produced a trend toward a decrease in body fat percent (-3.1%), and no changes in body weight. Despite the highly significant changes in body composition and body weight in the exercising group, preliminary results indicate no significant changes in serum estradiol or serum estrone. IGF-I did not change significantly either, indicating that chronic exercise and dieting do not result in favorable changes in two hormonal biomarkers for breast cancer. |   |  |   |                                  |
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## **INTRODUCTION**

This proposal entitled " Effects of moderate aerobic exercise combined with caloric restriction on circulating estrogens and IGF-I in premenopausal women" will provide important scientific contributions with respect to the primary prevention of breast cancer in women. Specifically, this ongoing study will examine potential mechanisms relating to the role of physical activity in the reduction of the risk of breast cancer by testing whether moderate aerobic exercise can reduced the levels of two hormonal biomarkers, circulating estrogens and insulin-like growth factor I (IGF-I). Since elevated levels of both of these hormones have been associated with an increased risk of breast cancer, and because exercise may modulate circulating levels, we wish to extend previous findings from epidemiological and cross-sectional studies by performing a tightly controlled, prospective clinical study that addresses previously unanswered questions related to the role of exercise in the modulation of estrogen and IGF-I. Although previous studies have shown that negative energy balance, and not other stressful aspects of physical exercise, can modulate reproductive function and therefore circulating estrogen levels, no studies to date have determined the magnitude of energy deficit required for these changes during long-term training, and no studies have attempted to differentiate between the exercise-induced changes in ovarian versus adipose sources of circulating estrogens. Since both estradiol (ovarian) and estrone (adipose tissue) are biologically active, and because the importance of estrone as a risk factor increases with age and adiposity, it is important to consider the degree to which exercise which creates a negative energy balance affects both of these sources of circulating estrogens.

Circulating levels of IGF-I correlate with breast cancer risk, yet studies examining the responses of this hormone and its binding proteins to chronic exercise are lacking. Since IGF-I levels are very sensitive to nutritional status, previously reported stimulatory effects of exercise on IGF-I can be overridden if exercise is performed in the face of negative energy balance. In this regard, exercise that promotes weight loss can be viewed as a way to reduce levels of IGF-I, and therefore potentially reduce the risk of breast cancers. To date, no studies have addressed whether a program of moderate aerobic exercise and dietary restriction producing a negative energy balance that is carried out over a long duration will significantly alter IGF-I levels. Further, the degree to which these levels might be altered in individuals of differing initial energy stores has not been addressed.

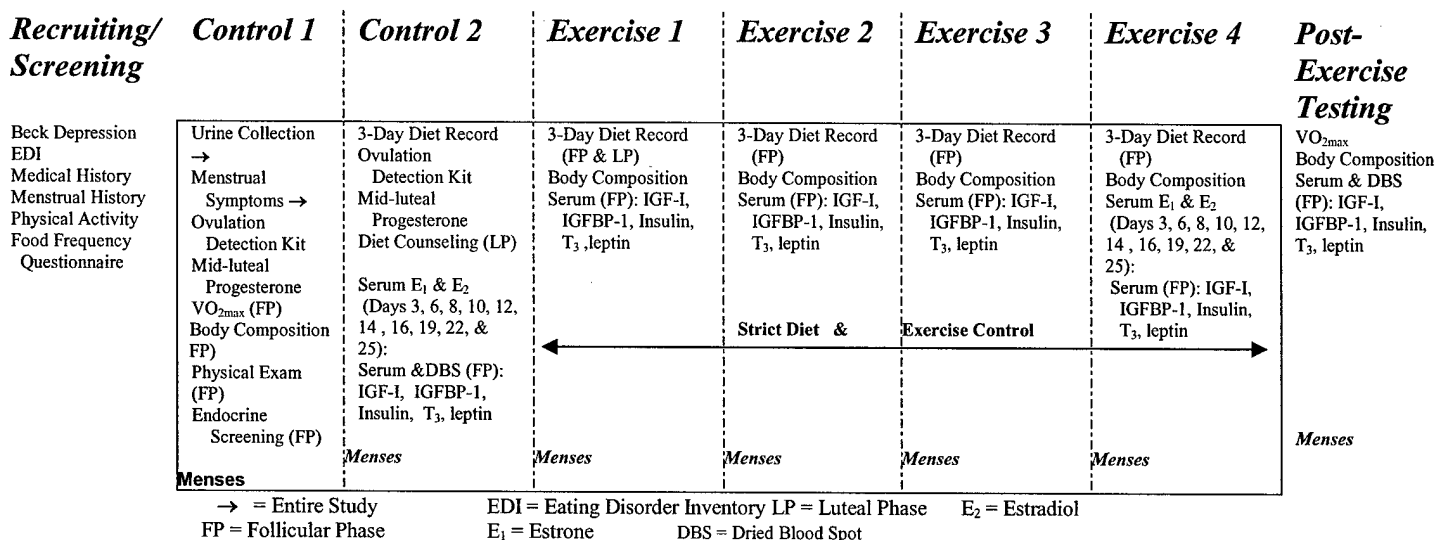
Metabolic energy availability is an important contributing factor in the development of reproductive cancers. However, current methods for assessing energy availability, which include anthropometric measures, calculations of energy balance, evaluation of various serum and urinary biomarkers are prone to measurement error, not sensitive to alterations in energy availability, and are sometimes affected by disease states. The current project includes an introduction of a novel approach to estimating energy status by measuring metabolic hormones in plasma, insulin, IGF-I, IGFBP-1 and leptin. Recently, dried blood spot (DBS) sample collection techniques have allowed for endocrine based population studies examining a wide variety of ecological factors that contribute to variation in human reproduction. In order to use the proposed method of energy status assessment in large population-based applications, such as those addressing the role of physical activity and or diet in the risk of breast cancer, the battery of metabolic hormones that comprise the proposed method must be amenable to collection and assays. Although the DBS technique has been partially validated for some hormonal assays, it has not yet been properly validated for insulin, IGF-I, IGFBP-1 and leptin, and it is unclear whether the technique is responsive to physiological changes of these compounds. Therefore, the current work calls for the validation of the DBS sampling technique for these assays under physiological conditions.

The proposed studies will yield new and important information regarding the degree to which an exercise and diet program that results in an energy deficit will reduce the risk of breast cancer.

## **BODY**

**Study Design:** The study utilizes a prospective, randomized design that tests the effects of a moderate exercise program (4X/wk; 4 months) combined with moderate dietary restriction that results in an average daily energy

deficit of -20%-30% kcals (Figure 1). Previously sedentary, eumenorrheic women aged 25-40 years will be assigned to exercise or light conditioning groups. Both normal weight (BMI 21-25 kg/m<sup>2</sup>) and overweight (BMI 26-30 kg/m<sup>2</sup>) will be assigned to either exercise or light conditioning group (exercise 2X/wk; no dietary restriction) groups; 4 groups, n=15 each group. Subjects will be studied for a total of six menstrual cycles, i.e., 2 control followed by 4 cycles with training and dietary restriction.



**Figure 1. Study Design**

### Progress According to the Approved Statement of Work:

(See previous Annual Summary for 2002-2003)

\*\*\*\*\*

#### Proposed Months 25-28

1. Repeat Steps above for year 3 recruiting and beginning testing (n=5 in each of 4 groups)
2. Perform assays on metabolic hormones in serum
3. Send serum and blood spot samples from year 2 subjects to DSL

*Actual Month 25, September, 2003:* Enrollment increased dramatically, fourfold increase in enrollment; assays completed, T3, and continued for IGF-1; arrangements made with Salimetrics Laboratory, University Park, PA to develop blood spot assays for Leptin, T3, and IGF-I (See Appendix); begun assays on urinary LH to document LH surges.

*Actual Month 26, October, 2003:* Continued with rolling recruitment, began screening procedures on recently enrolled subjects, completed IGF-I assays on completed subjects, continued with urinary LH assays to document LH surges

*Actual Month 27, November, 2003:* Continued with recruitment and testing, continued LH urinary assays

*Actual Month 28, December, 2003:* Continued with recruitment and testing, completed urinary LH assays on first cohort of completed subjects

#### Proposed Months 29-36:

1. Continue year 3 recruitment efforts only if necessary
2. Continue year 3 subject screening/initial testing

3. Complete year 3 subject exercise training/experimental testing
4. Perform urinary assays on LH, E3G, PdG urinary
5. Send serum and blood spot samples from year 3 subjects to DSL.
5. Perform data analysis and statistics

*Actual Month 29, January, 2004:* Continued testing and recruiting

*Actual Month 30, February, 2004:* Continued testing and recruiting

*Actual Month 31, March, 2004:* Continued testing and recruiting

*Actual Month 32, April, 2004:* Continued testing and recruiting

*Actual Month 33, May, 2004:* Continued testing and recruiting

*Actual Month 34, June, 2004:* Performed assays on next cohort of completed subjects for T3, leptin, and IGF-I; continued testing and recruiting.

*Actual Month 35, July, 2004:* Continued to perform assays for T3, leptin, and IGF-I. continued testing and recruiting; began database checking and data reduction; preliminary data analysis

*Actual Month 36, August, 2004:* Stopped recruitment of subjects, continued with testing of currently enrolled subjects; Finished assays for T3, leptin, and IGF-I for most recently finished cohort. Performed assays for estradiol for most recently completed cohort.

*Actual Month 37, September, 2004:* Continued with testing, preliminary data analysis and database management; perform preliminary statistics for annual report; examine results from Salimetrics thus far

Newly Proposed Months 38-42, October, 2004-February, 2005:

Request extension for final report for this study (IDEA Award) from DAMD; perform urinary LH, E1G, and PDG assays, and serum metabolic assays when final cohort is finished; perform insulin, IGFBP-2, estradiol, and estrone on remaining completed subjects; send completed DBS samples from completed subjects to Salimetrics; perform data analysis; obtain results of DBS samples from Salimetrics, submit abstract for ERA of HOPE Meeting in December, 2004

Newly Proposed Months 43-48, March 2005- May 2005:

Write and submit manuscripts

### **Preliminary Results From Years 1, 2, and 3:**

#### **Subject Recruitment:**

We have accumulated approximately 269 phone and e-mail contacts since September 03, bringing our 3 year total number of contacts up to 571. In the last 3 years seventy-eight women have begun the study and 52 have dropped out or been excluded for the following reasons: 14 with menstrual abnormality detected in the control months, 10 for medical reasons, 21 self-drop-out (time, intervention), 2 non-compliance, 2 with pregnancies during the study, and 3 because of weight/body composition measures outside inclusion criteria during screening. Although this represents a high drop-out rate (66%) prior to screening, our drop-out rate after screening procedures have been performed (including two control menstrual cycles to determine normal menstrual cyclicity) is 52%. That is, 52% of the women that made it through out screening procedures have dropped out of the study. This rate is much higher than predicted (20%).

Our recruitment efforts were stepped up beginning September 2003. Although we generally see do well with inquiries about the study, we had an especially disheartening recruitment effort this year, as our dropout rate

was double what it has been in the past two years. Therefore, our current subject numbers are reflected in Table 1. Descriptive characteristics of these subjects are shown in Table 2.

**Table 1. Current Enrollment and Completed Subjects**

| Experimental Group          | Initially Began Study After Screening<br>(N) | Currently Enrolled<br>(N) | Finished Study<br>(N) | Potential Final<br>(N) |
|-----------------------------|--|---------------------------|-----------------------|------------------------|
| Light Conditioning-low BMI  | 8  | 0                         | 6                     | 6                      |
| Light Conditioning-high BMI | 6  | 1                         | 2                     | 3                      |
| Exercisers-low BMI          | 20   | 3                         | 14                    | 17                     |
| Exercisers-high BMI         | 11   | 2                         | 5                     | 7                      |

**Table 2. Initial Characteristics of Subjects Completed or Currently Enrolled in the Study**

| Variable                       | Light Conditioning-low BMI (LCLB) | Light Conditioning-high BMI (LCHB) | Exercisers-low BMI (ELB)  | Exercisers-high BMI (EHB)   |
|--------------------------------|-----------------------------------|------------------------------------|---------------------------|-----------------------------|
| Age (yrs)                      | 33.63 ± 3.34                      | 34.83 ± 4.26                       | 32.00 ± 4.75              | 31.73 ± 2.90                |
| Weight (kg)                    | 61.43 ± 3.70                      | 68.88 ± 3.73                       | 60.05 ± 6.12 <sup>d</sup> | 73.56 ± 9.04 <sup>c,f</sup> |
| Height (cm)                    | 166.46 ± 5.27                     | 160.97 ± 6.88                      | 163.64 ± 5.90             | 163.08 ± 6.73               |
| BMI (kg/m <sup>2</sup> )       | 22.21 ± 1.93                      | 27.35 ± 2.16 <sup>a</sup>          | 22.27 ± 1.75 <sup>d</sup> | 27.95 ± 2.09 <sup>c,f</sup> |
| Body Fat (%)                   | 31.10 ± 4.03                      | 39.20 ± 4.07 <sup>a</sup>          | 29.96 ± 3.50 <sup>d</sup> | 37.78 ± 4.62 <sup>c,f</sup> |
| VO <sub>2</sub> Max (mg/kg/ml) | 30.36 ± 1.99                      | 26.43 ± 4.14                       | 33.88 ± 5.19 <sup>d</sup> | 27.63 ± 6.43 <sup>f</sup>   |

One-way ANOVA; Post-hoc: LSD; P<0.05

<sup>a</sup>LCLB vs LCHB

<sup>b</sup>LCLB vs ELB

<sup>c</sup>LCLB vs EHB

<sup>d</sup>LCHB vs ELB

<sup>e</sup>LCHB vs EHB

<sup>f</sup>ELB vs EHB

### Preliminary Results:

**Aerobic Capacity, Body Weight and Body Composition:** Our light conditioning group exhibited a trend toward a decrease in percent body fat of -3.1% (P< 0.072), but no significant changes in body weight or BMI. The exercising group experienced significant declines in both body weight (-6.2%) and percent body fat (-15.7% of initial percent fat), fat mass (-20.6%) and in BMI (-6.8%). Both groups significantly increased their aerobic capacity, i.e., Light conditioning increased by 33% and exercising group increased by 28% (Tables 3 and 4).

**Estradiol and Estrone:** When serum measurements of these hormones across Control Cycle 2 (n=10), and Exercise 4 cycles (n=10) are averaged, and then compared with paired samples T-tests, no differences are observed in either the light conditioning or exercising groups, despite the loss of body fat (Tables 3 and 4). A

composite graph of these changes, depicted according to cycle day is illustrated in Figure 2. A representative depiction of the changes in both serum estrone and estradiol and urinary E1G is depicted in Figure 3.

**Table 3. Paired-Samples T-Tests Comparing Pre to Post Intervention in Light Conditioning Group**

**Paired Samples Statistics**

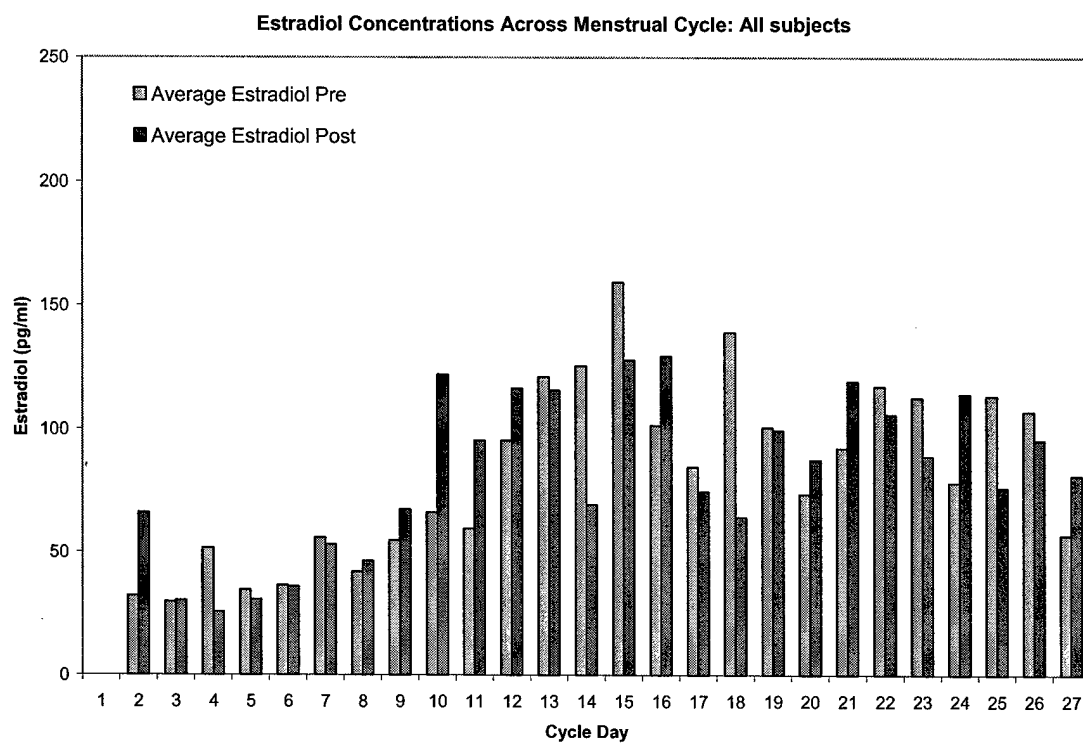
|        |  | Mean    | N | Std. Deviation | Std. Error Mean | Sig. (2-tailed) |
|--------|--|---------|---|----------------|-----------------|-----------------|
| Pair 1 | Pre VO2 Max (ml/kg/min)                                | 28.3500 | 6 | 3.56693        | 1.45619         | .001            |
|        | Post VO2 Max (ml/kg/min) ( Ex 3 BIOEY1; Post BIOEY2&3) | 37.7667 | 6 | 4.17069        | 1.70268         |                 |
| Pair 2 | Pre UWW Weight (kg) (control month BIOEY 1-3)          | 63.1429 | 7 | 4.37516        | 1.65365         | .114            |
|        | Post UWW Weight (kg)(Ex 3 BIOEY 1; Post BIOEY 2&3)     | 62.3071 | 7 | 4.62911        | 1.74964         |                 |
| Pair 3 | Pre UWW % Body Fat (control month BIOEY 1-3)           | 33.4514 | 7 | 4.71277        | 1.78126         | .072            |
|        | Post UWW % Body Fat(Ex 3 BIOEY1; Post BIOEY2&3)        | 32.2000 | 7 | 5.53164        | 2.09076         |                 |
| Pair 4 | Pre UWW BMI (control month BIOEY 1-3)                  | 23.4186 | 7 | 3.52394        | 1.33193         | .104            |
|        | Post UWW BMI (Ex 3 BIOEY1; Post BIOEY2&3)              | 23.1386 | 7 | 3.56102        | 1.34594         |                 |
| Pair 5 | Pre Month RMR (kcal/min)                               | .9045   | 6 | .06965         | .02844          | .066            |
|        | Post Exercise Month RMR (kcal/min)                     | .9778   | 6 | .05128         | .02094          |                 |
| Pair 6 | EstradiolAvePRE  | 70.3756 | 5 | 13.53968       | 6.05513         | .161            |
|        | EstradiolAVERAGEPOST                                   | 80.4066 | 5 | 16.36771       | 7.31986         |                 |
| Pair 7 | Pre UWW Ave FFmass (kg)(control month BIOEY 1-3)       | 41.8900 | 7 | 2.10473        | .79551          | .146            |
|        | Post UWW Ave FFmass (kg) (Ex 3 BIOEY1; Post BIOEY2&3)  | 42.0729 | 7 | 2.25627        | .85279          |                 |
| Pair 8 | Pre UWW Ave Fatmass (kg) (control month BIOEY 1-3)     | 21.1643 | 7 | 4.29164        | 1.62209         | .107            |
|        | Post UWW Ave Fatmass (kg) (Ex 3 BIOEY1; Post BIOEY2&3) | 20.2343 | 7 | 4.82436        | 1.82344         |                 |



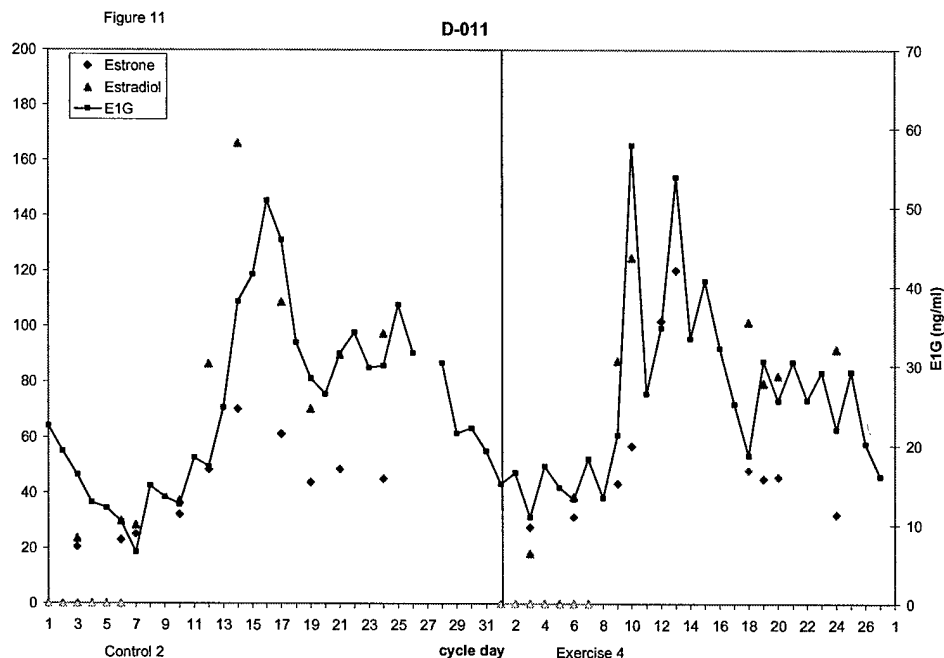
**Table 4. Paired-Samples T-Tests Comparing Pre to Post Intervention in Exercising Group**

**Paired Samples Statistics**

|        |   | Mean    | N  | Std. Deviation | Std. Error<br>Mean | Sig. (2-<br>tailed) |
|--------|---|---------|----|----------------|--------------------|---------------------|
| Pair 1 | Pre VO2 Max<br>(ml/kg/min)                                      | 32.9938 | 16 | 5.36675        | 1.34169            |                     |
|        | Post VO2 Max<br>(ml/kg/min) ( Ex 3<br>BIOEY1; Post<br>BIOEY2&3) | 42.1938 | 16 | 9.80064        | 2.45016            | .001                |
| Pair 2 | Pre UWW Weight (kg)<br>(control month BIOEY<br>1-3)             | 63.7590 | 20 | 9.50026        | 2.12432            |                     |
|        | Post UWW Weight<br>(kg)(Ex 3 BIOEY 1;<br>Post BIOEY 2&3)        | 59.7575 | 20 | 9.03649        | 2.02062            | .000                |
| Pair 3 | Pre UWW % Body Fat<br>(control month BIOEY<br>1-3)              | 31.6030 | 20 | 5.20520        | 1.16392            |                     |
|        | Post UWW % Body<br>Fat(Ex 3 BIOEY1; Post<br>BIOEY2&3)           | 26.6530 | 20 | 6.28069        | 1.40440            | .000                |
| Pair 4 | Pre UWW BMI (control<br>month BIOEY 1-3)                        | 23.5036 | 20 | 3.09325        | .69167             |                     |
|        | Post UWW BMI (Ex 3<br>BIOEY1; Post<br>BIOEY2&3)                 | 21.9395 | 20 | 2.66114        | .59505             | .000                |
| Pair 5 | Pre Month RMR<br>(kcal/min)                                     | .9231   | 20 | .09858         | .02204             |                     |
|        | Post Exercise Month<br>RMR (kcal/min)                           | .8964   | 20 | .11824         | .02644             | .146                |
| Pair 6 | EstradiolAvePRE   | 95.0502 | 12 | 21.46584       | 6.19665            |                     |
|        | EstradiolAVERAGEPO<br>ST  | 93.3349 | 12 | 28.63159       | 8.26523            | .828                |
| Pair 7 | Pre UWW Ave FFmass<br>(kg)(control month<br>BIOEY 1-3)          | 43.3275 | 20 | 5.13945        | 1.14922            |                     |
|        | Post UWW Ave<br>FFmass (kg) (Ex 3<br>BIOEY1; Post<br>BIOEY2&3)  | 43.5390 | 20 | 5.48725        | 1.22699            | .460                |
| Pair 8 | Pre UWW Ave Fatmass<br>(kg) (control month<br>BIOEY 1-3)        | 20.4070 | 20 | 5.92869        | 1.32570            |                     |
|        | Post UWW Ave<br>Fatmass (kg) (Ex 3<br>BIOEY1; Post<br>BIOEY2&3) | 16.2185 | 20 | 5.47167        | 1.22350            | .000                |
| Pair 9 | EstroneAVERAGEPRE   | 58.7406 | 7  | 15.66615       | 5.92125            |                     |
|        | EstroneAVERAGEPOS<br>T  | 56.7727 | 7  | 13.95731       | 5.27537            | .765                |



**Figure 2. Composite graph of estradiol measurements from Control Cycle 2 (Pre) and Exercise 4 (Post) cycles.**



**Figure 3. Representative example of a single subject's urinary (E1G) and serum estrogens (estrone and estradiol) before (Control 2) and after (Exercise 4) exercise training combined with caloric restriction.**

Serum Leptin, T3, and IGF-I: A significant decrease ( $P < 0.05$ ) was observed in measurements for leptin (Table 5), primarily accounted for by the changes in the exercising group ( $P < 0.05$  group X time interaction). Serum T3 decreased significantly overall ( $P < 0.05$ ) (Table 6). No significant differences were observed in either group for IGF-I (Table 7).

**Table 5. Serum Leptin for Light Conditioning (0) and Exercisers (1) Pre and Post study**

|                     | Groups based on exercise | Mean    | Std. Deviation | N  |
|---------------------|--------------------------|---------|----------------|----|
| Pre Leptin (ng/ml)  | .00                      | 13.0750 | 6.40459        | 6  |
|                     | 1.00                     | 15.9233 | 11.82781       | 18 |
|                     | Total                    | 15.2113 | 10.67270       | 24 |
| Post Leptin (ng/ml) | .00                      | 15.0050 | 9.24739        | 6  |
|                     | 1.00                     | 7.4656  | 7.21061        | 18 |
|                     | Total                    | 9.3504  | 8.25476        | 24 |

**Table 6. Serum T3 for Light Conditioning (0) and Exercisers (1) Pre and Post study**

|                       | Groups based on exercise | Mean     | Std. Deviation | N  |
|-----------------------|--------------------------|----------|----------------|----|
| Total T3 Pre (ng/dl)  | .00                      | 107.0380 | 24.17636       | 5  |
|                       | 1.00                     | 104.0635 | 14.02287       | 17 |
|                       | Total                    | 104.7395 | 16.21055       | 22 |
| Total T3 Post (ng/dl) | .00                      | 96.1460  | 22.05372       | 5  |
|                       | 1.00                     | 95.1106  | 16.04316       | 17 |
|                       | Total                    | 95.3459  | 16.99823       | 22 |

**Table 7. Serum IGF-I for Light Conditioning (0) and Exercisers (1) Pre and Post study**

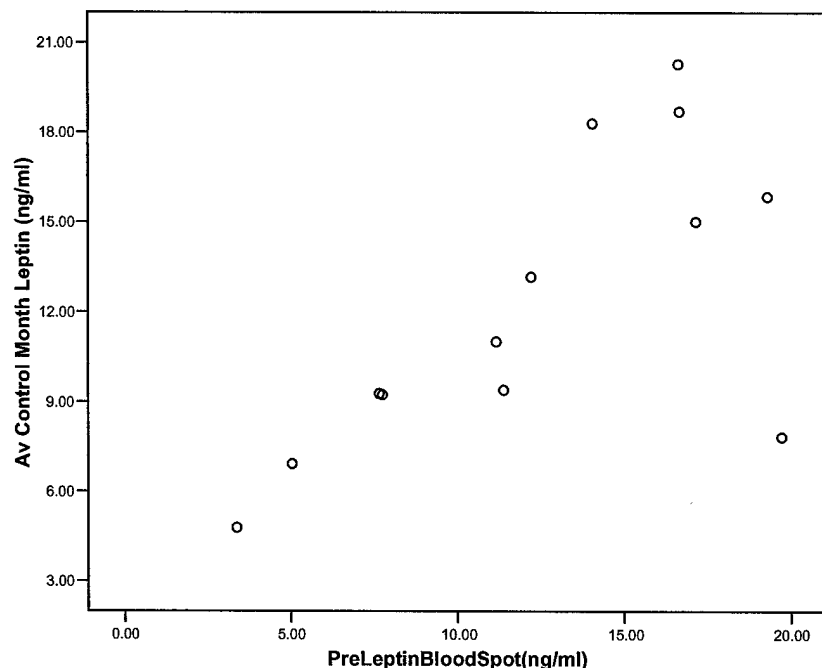
|                    | Groupsbasedonexercise | Mean     | Std. Deviation | N  |
|--------------------|-----------------------|----------|----------------|----|
| IGF-1Pre (ng/ml)   | .00                   | 238.3740 | 56.80587       | 5  |
|                    | 1.00                  | 211.4859 | 50.48205       | 17 |
|                    | Total                 | 217.5968 | 51.85874       | 22 |
| IGF-1 Post (ng/ml) | .00                   | 230.6080 | 26.58635       | 5  |
|                    | 1.00                  | 203.6382 | 58.94881       | 17 |
|                    | Total                 | 209.7677 | 54.00045       | 22 |

Results for Dried Blood Spot Samples: Thus far, Salimetrics has provided us with results for leptin DBS samples. In comparison to the simultaneous venipuncture measurement of leptin as assayed in our laboratory, a significant correlation exists ( $P < 0.05$ ; Pearson Correlation) (Table 8, and Figure 4)

**Table 8. Correlation between serum and DBS sample for leptin**

|                                    |                     | Av Control<br>Month Leptin<br>(ng/ml) | PreLeptinBlood<br>Spot(ng/ml) |
|------------------------------------|---------------------|---------------------------------------|-------------------------------|
| Av Control Month<br>Leptin (ng/ml) | Pearson Correlation | 1                                     | .677(*)                       |
|                                    | Sig. (2-tailed)     | .                                     | .011                          |
|                                    | N                   | 36                                    | 13                            |
| PreLeptinBloodSpot<br>(ng/ml)      | Pearson Correlation | .677(*)                               | 1                             |
|                                    | Sig. (2-tailed)     | .011                                  | .                             |
|                                    | N                   | 13                                    | 13                            |

\* Correlation is significant at the 0.05 level (2-tailed).



**Figure 4. Scatterplot of leptin DBS vs venipuncture results**

Overall Results from Years 1-3: Although our data set is not yet complete, it appears as though there are no dramatic changes in circulating estradiol, estrone, or in urinary E1G in our subjects, despite significant loss of weight and decrease in body fat. Preliminary results comparing DBS technique to venipuncture as assayed by RIA are very promising.

## **KEY ACCOMPLISHMENTS**

This is an ongoing study, so preliminary publication of the data is not feasible.

## **REPORTABLE OUTCOMES**

### Training:

The following individuals have been supported by DAMD17-01-1-0360:

#### Faculty:

Nancy Williams, Sc.D.

#### Undergraduate Kinesiology Students:

Carmon Communale 3/04-5/04

Kristin Gross 2/04-9/04

The following students received degrees in the past year under the direction of Dr. Williams. These students all assisted with the current project.

Kelly Dougherty, MS Kinesiology

Brian Frye, MS Kinesiology

Publications:

To date, no publications have resulted from the project supported by these funds because the data set is not yet complete. However, Dr. Williams has produced the following publications while being supported by this Career Award:

Published Manuscripts:

**Williams, NI.** Experimental disruptions of the menstrual cycle: Lessons from long-term prospective studies. Med Sci Sports Exerc 35 (8): 1564-1572, 2003.

De Souza, M.J., H. McConnell, E. O'Donnell, B. Lasley, and **Williams NI.** Fasting Ghrelin Levels in Physically Active Women: Relationship with Menstrual Disturbances and Metabolic Status. J Clin Endocrinol Metab. Jul;89(7):3536-42, 2004.

McConnell HJ, Gardner JK, Frye BR, Snook ML, Schuchert MK, Richard EL, and **Williams NI.** Circulating ghrelin is sensitive to changes in body weight during a diet and exercise program in normal weight young women (Special edition: J Clin Endocrinol Metab. Jun;89(6):2659-64, 2004.

De Souza, M.J., and **N.I. Williams.** Physiological Aspects and Clinical Sequelae of Energy Deficiency and Hypoestrogenism in Exercising Women. Hum Reprod Update. Sep-Oct;10(5):433-48, 2004

**Williams, NI** and De Souza, MJ. "Exercise-associated menstrual cycle disturbances: practical and clinical considerations", Endocrinology of Physical Exercise and Sport as part of the series "The Encyclopedia of Sports Medicine" for the International Olympic Committee (In Press, for 2005).

Manuscripts in Review:

De Souza MJ, and **Williams NI.** Beyond Hypoestrogenism in Amenorrheic Athletes: Energy Deficiency as a Contributing Factor for Bone Loss (Submitted to Current Sports Medicine Reports)

Mastro AM, Williams NI, Kraemer WJ et al. Exercise Intervention and Plasma Levels of IFN-, and IL-6 following Chemotherapy for Breast Cancer. (submitted to the Journal of Clinical Oncology).

Manuscripts in Progress:

Williams NI, Perry MD, Kraemer WJ, and Mastro AM. "Effects of chemotherapy followed by exercise training on reproductive status and stress hormones in breast cancer patients"

**Williams, N.I. Williams, N.I.**, Berga S.L., and Cameron, J.L. Synergism of multiple sub-threshold stressors: effects of diet, exercise, and psychosocial stress on menstrual cyclicity

**Leidy HJ**, Frye BR, Duke KM, Albert AE, Snook ML, **Williams NI.** Changes in ghrelin are concomitant with changes in body weight, leptin, and IGF-1 during an energy deficit-imposing diet and exercise program in normal weight, healthy young women

**Leidy HJ**, Frye BR, Duke KM, Albert AE, Snook ML, **Williams NI.** Meal Calorie Content and Meal Timing Affect Specific Meal Response Characteristics of Total Ghrelin in Normal Weight Healthy Young Women

**Leidy HJ, Frye BR, Duke KM, Albert AE, Snook ML, Williams NI.** The Meal Related Pattern and Diurnal Rhythm of Ghrelin are Elevated Following an Energy Deficit-imposing Diet and Exercise Intervention

Cancer Grants:

Active: The following grant was awarded for additional studies to be undertaken in collaboration with Dr. Kimberly Westerlind at the AMC Cancer Research Center in Denver, Colorado. This work will examine the effects of exercise on the ratios of urinary estrogen metabolites.

AMC Cancer Research Center

**Co-Investigator (Williams)**

**PI (Westerlind)**

1/04-12/05

\$76,865

"Exercise and Estrogen Metabolism: Implications for Breast Cancer Prevention"

Pending: The principal investigator plans to submit an NIH R01 grant to secure funding to perform additional analyses on serum samples collected from the current project. This grant will be in response to PA-04-124 (Studies of Energy Balance and Cancer in Humans) July 7, 2004 -Sept 2, 2006.

"Effects of energy deficiency on hormonal and immunological biomarkers for cancer"

Other Grants:

(Active Support)

**1. NIH**

1 RO1 HD39245-01 (Williams)

5/1/01 - 4/30/04

(currently in 1 yr no cost extension) 30%

PHS/NICHD

\$ 1,538,361

**Principal Investigator:**

"Bioenergetics of Exercise-induced Menstrual Disturbances"

**2. US Army Medical Research and Materiel Command**

9/17/01- 9/16/05 15%

US Army Breast Cancer Program (IDEA AWARD)

\$408,878

**Principal-Investigator:**

"Effects of Moderate Aerobic Exercise Combined with Caloric Restriction on Circulating Estrogens and IGF-1 in Premenopausal Women (IDEA Award) "

**3. US Army Medical Research and Materiel Command**

9/17/01- 9/16/05 50%

US Army Breast Cancer Program

\$312,081

(CAREER DEVELOPMENT AWARD)

**Principal-Investigator:**

"Effects of Moderate Aerobic Exercise Combined with Caloric Restriction on Circulating Estrogens and IGF-1 in Premenopausal Women (Salary Only)

**4. Retirement Research Foundation**

2000-2004

2%

**\$56,832****Co-Investigator:** (PI is J.L. Cameron, PhD)

"Physical Exercise and Brain Aging"

**5. National Institutes of Health (NIH)**

2003-2008

HD-02-012 Cooperative Reproductive Science Research Centers at Minority Institutions

**Co-Investigator:**

\$ 1,160,204

5%

"The efficacy and safety of metformin and lifestyle factors in the amelioration of hyperandrogenemia and its associated symptomology"

**6. Cancer Research and Prevention Foundation****Co-Investigator (PI is Kim Westerlind, AMC Cancer Research Center, Denver, CO)**

1/04-12/05 0%

**\$76,865**

"Exercise and Estrogen Metabolism: Implications for Breast Cancer Prevention"

**7. NASA****Co-Investigator (PI is James Pawelczyk, PSU)**

4/1/05-3/31/06

5%

**\$1,144,613**

"Improving Orthostatic Tolerance in Women: Control of Splanchnic and Cutaneous Vascular Capacitance"

\*\*\*\*\*

**(Pending)****1. National Institutes of Health (NIH)**

4/1/05-3/31/09

28.8%

Co-Principal Investigator (PI is Susan Bloomfield, Texas A&amp;M)

**\$2,000,000**

"Impact of Food Restriction on Bone Health in Active Females"

\*\*\*\*\*

**(Not funded)****1. National Institutes of Health (NIH)**

1 RO1 (Co -Principal Investigator with Mary Jane De Souza, Univ. Toronto)

7/01/02 - 6/30/07

15%

PHS/NICHD

**\$ 2,433,044**

"Clinical Sequelae Exercise-Induced Hypoestrogenism"

**2. National Insitutes of Health (NIH)****Co-Investigator (PI is Terryl Hartman, PSU)**

4/01/04-3/31/08

20%

**\$2,085,448**

"Female Cancer Survivors Weight and Activity Intervention"

**3. Dairy Farmers of Canada**

1/1/05-12/31/06

10%

Co- Principal Investigator (PI is Mary Jane De Souza, University of Toronto)



**“Can Increased Dietary Calcium Improve Recovery of Bone Health in Exercising Women Undergoing a Lifestyle Intervention for Severe Menstrual Disturbances?”**

**4. National Institutes of Health (NIH)**

4/1/05- 3/31/09

15%

Co-Investigator (PI is Terry Hartmen, Dept. Nutrition, Penn State)

\$856,295

**“Antioxidant Status, Diet and Early Pregnancy”**

**Presentations:**

J K Gardner, H J McConnell, B R Frye, K A Dougherty, T S Parrott, E L Richard, M L Snook, M Schukert, **Williams, NI.** Validation of an Improved Method to Estimate Energy Requirements in College-aged Women: The PERK method (*Proceedings of the American College of Sports Medicine meeting, Indianapolis, IN, 2004 Med. Sci. Sports Exerc.* 36 (5), p. S79, 2004)

K Dougherty, H J McConnell, J Gardner, B Frye, E Richard, M Snook, M Schucert, A Albert, T Parrott and **Williams, NI.** Effects of Diet and Exercise on Leptin Levels In Women: Dependence on Body Composition Changes (*Proceedings of the American College of Sports Medicine meeting, Indianapolis, IN, 2004 Med. Sci. Sports Exerc.* 36 (5), p. S80, 2004)

**Williams, NI,** HJ McConnell, JK Gardner, BR Frye, EL Richard, ML Snook, KL Dougherty, TS Parrott, A Albert, M. Schukert. Exercise-associated menstrual disturbances: dependence on daily energy deficit, not body composition or body weight changes. (*Proceedings of the American College of Sports Medicine meeting, Indianapolis, IN, 2004 Med. Sci. Sports Exerc.* 36 (5), p. S280, 2004.

De Souza, M.J., E. O'Donnell, R. Hontscharuk, T. Burke, J. Goodman, **Williams, NI.** Diagnosis of Osteopenia May Indicate the Presence of Increased Cardiovascular Risk in Female Athletes. (*Proceedings of the American College of Sports Medicine meeting, Indianapolis, IN, 2004 Med. Sci. Sports Exerc.* 36 (5), p. S280, 2004.

R. Hontscharuk, E. O'Donnell, **Williams, NI** T. Burke, M.J. De Souza. Dietary Cognitive Restraint: A Marker for Altered Energy Homeostasis and Menstrual Disturbances in Athletic Women (*Proceedings of the American College of Sports Medicine meeting, Indianapolis, IN, 2004 Med. Sci. Sports Exerc.* 36 (5), p. S33, 2004.

McConnell, H., **Williams, NI,** E. O'Donnell, B. Lasley, M.J. De Souza. Fasting Ghrelin Levels in Physically Active Women: Relationship with Menstrual Disturbances and Metabolic Status. (*Proceedings of the American College of Sports Medicine meeting, Indianapolis, IN, 2004 Med. Sci. Sports Exerc.* 36 (5), p. S280, 2004)

**CONCLUSIONS:**

We are making good progress toward the completion of this study. Preliminary examination of the data look interesting but thus far analyses do not support the hypothesis that circulating biomarkers of breast cancer are altered by diet and exercise. The DBS technique looks promising as a potential field marker for energy availability, based on a favorable correlation between serum and DBS samples.

**REFERENCES:**

NONE

**APPENDIX**

1. Letter from Salimetrics
2. T3 performance and quality control characteristics
3. IGF-I performance and quality control characteristics

Dr. Nancy I. Williams  
Associate Professor  
Room 267Q  
Recreation Building  
Department of Kinesiology  
& Noll Physiological Research Center  
Penn State University  
University Park, PA 16802

Dear Dr. Williams,

At Dr. Granger's request, I am writing to outline the basic objectives for our development of blood spot assays for Total T3 and IGF-1 for your project. You should have received quotes for these projects from Martha Orland last weekend. As you are aware, we have already developed similar assays for testosterone, leptin, estradiol, and progesterone. Based on our previous experience I don't expect protocol development for these markers will be problematic. Nevertheless, as with any research project a specific timeline is difficult to predict. We hope the development work will take no longer than 3 months time.

As in the past, our approach will be to begin by modifying commercially available enzyme immunoassay protocols. The assay development will include determination of assay range, lower limit of sensitivity, linearity and spike recovery, and confirmation that intra- and inter-assay coefficients of variation are within acceptable limits outlined by Chard (1990). We will also provide recommendations regarding sample collection, preparation, and the amount of sample needed to perform each assay.

In a previous note to Dr. Granger, you mentioned having matched serum/plasma samples. Once the assay is internally validated we highly recommend comparing values from the blood spot assay protocols with results you obtain from the serum tests. We can arrange those serum tests for you if you don't already have a source for those assays.

Once completed, we can provide testing services for your project at a cost of \$25.00 per sample for T3 and >\$30.00 per sample for IGF-1.

If you have any questions or are just interested in a progress report, please don't hesitate to call (800-790-2258 ext. 207) or email me ([Ebs@salimetrics.com](mailto:Ebs@salimetrics.com)).

Best Regards,

Eve Schwartz



## T3 BLOOD SPOT PERFORMANCE CHARACTERISTICS

### LINEARITY OF DILUTION :

A plasma sample was diluted linearly and each dilution was mixed with equal parts of RBCs. 50 ul of each mixture was pipetted onto blood spot papers, frozen, thawed, and then assayed.

| DILUTION<br>FACTOR | EXPECTED<br>ng/dL | OBSERVED<br>ng/dL | RECOVERY<br>% |
|--------------------|-------------------|-------------------|---------------|
| 0                  |                   | 287.75            |               |
| x2                 | 143.88            | 135.20            | 94.0%         |
| x4                 | 71.94             | 77.84             | 108.2%        |

### PRECISION:

The intra-assay precision was determined from the mean of 10 replicates each.

| SAMPLE | N  | MEAN<br>ng/dL | STANDARD<br>DEVIATION ng/dL | COEFFICIENT OF<br>VARIATION % |
|--------|----|---------------|-----------------------------|-------------------------------|
| C I    | 10 | 140.32        | 12.45                       | 8.9                           |
| C II   | 10 | 70.07         | 4.43                        | 6.3                           |

The inter-assay precision was determined from the mean of average duplicates for 4 separate runs.

| SAMPLE | N | MEAN<br>ng/dL | STANDARD<br>DEVIATION ng/dL | COEFFICIENT OF<br>VARIATION % |
|--------|---|---------------|-----------------------------|-------------------------------|
| C I    | 4 | 146.65        | 8.71                        | 5.9                           |
| C II   | 4 | 64.73         | 6.42                        | 9.9                           |

### **SPIKE AND RECOVERY:**

The zero calibrator was spiked with three different levels of T3 and mixed with equal parts of RBCs. 50 ul of each mixture was pipetted onto blood spot papers, frozen, thawed, and then assayed.

| Endogenous<br>(ng/dL) | Added<br>(ng/dL) | Expected<br>(ng/dL) | Observed<br>(ng/dL) | Recovery<br>(%) |
|-----------------------|------------------|---------------------|---------------------|-----------------|
| 0                     | 37.5             | 37.5                | 34.7                | 92.5            |
| 0                     | 112.5            | 112.5               | 94.9                | 84.3            |
| 0                     | 187.5            | 187.5               | 157.5               | 84.0            |

### **SENSITIVITY:**

The low limit of sensitivity of the assay was determined by mixing equal parts of the zero calibrator and red blood cells and spotting 50 uL onto blood spot papers. The spots were frozen and thawed before assay. The lower limit of sensitivity was determined by interpolating the mean minus 2 SD for eleven zeros. The minimal concentration of T3 that can be distinguished from 0 is 30 ng/dL



## IGF-1 BLOOD SPOT PERFORMANCE CHARACTERISTICS

### LINEARITY OF DILUTION :

A plasma sample was diluted linearly and each dilution was mixed with equal parts of RBCs. 50 ul of each mixture was pipetted onto blood spot papers, frozen, thawed, and then assayed.

| DILUTION<br>FACTOR | EXPECTED<br>ng/mL | OBSERVED<br>ng/mL | RECOVERY<br>% |
|--------------------|-------------------|-------------------|---------------|
| 0                  |                   | 270.17            |               |
| x2                 | 135.09            | 133.04            | 98.5          |
| x4                 | 67.54             | 57.27             | 84.8          |

### PRECISION:

The intra-assay precision was determined from the mean of 10 replicates of low, mid and high concentrations of IGF-1.

| SAMPLE | N  | MEAN<br>ng/mL | STANDARD<br>DEVIATION ng/mL | COEFFICIENT OF<br>VARIATION % |
|--------|----|---------------|-----------------------------|-------------------------------|
| Low    | 10 | 25.0          | 1.75                        | 7.0                           |
| Mid    | 10 | 59.14         | 3.19                        | 5.4                           |
| High   | 10 | 129.96        | 8.87                        | 6.8                           |

The inter-assay precision was determined from the mean of average duplicates for 4 separate runs.

| SAMPLE | N | MEAN<br>ng/mL | STANDARD<br>DEVIATION ng/mL | COEFFICIENT OF<br>VARIATION % |
|--------|---|---------------|-----------------------------|-------------------------------|
| Low    | 4 | 93.13         | 4.44                        | 4.8                           |
| High   | 4 | 166.85        | 7.55                        | 4.5                           |

### **SPIKE AND RECOVERY:**

The zero calibrator was spiked with three different levels of IGF-1 and mixed with equal parts of RBCs. 50 ul of each mixture was pipetted onto blood spot papers, frozen, thawed, and then assayed.

| Endogenous<br>(ng/mL) | Added<br>(ng/mL) | Expected<br>(ng/mL) | Observed<br>(ng/mL) | Recovery<br>(%) |
|-----------------------|------------------|---------------------|---------------------|-----------------|
| 0                     | 55.0             | 55.0                | 64.3                | 116.9           |
| 0                     | 110.0            | 110.0               | 117.1               | 106.5           |
| 0                     | 165              | 165                 | 158.7               | 96.2            |

### **SENSITIVITY:**

The low limit of sensitivity of the assay was determined by mixing equal parts of the zero calibrator and red blood cells and spotting 50 uL onto blood spot papers. The spots were frozen and thawed before assay. The lower limit of sensitivity was determined by interpolating the mean minus 2 SD for 10 zeros. The minimal concentration of IGF-1 that can be distinguished from 0 is < 20 ng/mL